

WE CLAIM:

1. A method of detecting the activity of an enzyme that operates on an enzyme substrate to form an enzyme product in a sample, comprising:

5 contacting the substrate with a binding partner that specifically binds to the substrate or to the product but not to both, where the binding partner includes a metal ion that is required for binding between the binding partner and the substrate or product;

contacting the substrate with the enzyme;

10 detecting a response indicative of the extent of binding between the substrate or the product and the binding partner without separating the bound substrate or product from the unbound substrate or product; and

correlating the response with the activity of the enzyme.

15 2. The method of claim 1, where the metal ion is a tricationic metal ion.

3. The method of claim 2, where the tricationic metal ion is selected from the group consisting of Al^{3+} , Fe^{3+} , and Ga^{3+} .

4 The method of claim 3, where the tricationic metal ion is Ga^{3+} .

20 5. The method of claim 2, where the binding partner further includes a dicationic metal ion.

6. The method of claim 1, where the step of detecting a response comprises:
exposing the sample to a condition capable of inducing luminescence from the
sample; and

measuring a detectable luminescence response, where the detectable luminescence
5 response is indicative of the extent of binding between the substrate or product and the
binding partner.

7. The method of claim 6, where at least one of the substrate, product, and
binding partner is luminescent.

8. The method of claim 6, where the condition is light capable of inducing
photoluminescence.

9. The method of claim 8, where the detectable luminescence response is
luminescence intensity.

10. The method of claim 8, where the detectable luminescence response is
luminescence polarization.

11. The method of claim 8, where the detectable luminescence response is
luminescence resonance energy transfer.

12. The method of claim 6, where the condition is electrochemical energy capable of inducing electrochemiluminescence.

13. The method of claim 1, where the substrate is a polypeptide, and where the
5 substrate and product are related by a posttranslational modification.

14. The method of claim 13, where the posttranslational modification is phosphorylation or dephosphorylation of the polypeptide.

15. The method of claim 1, where the substrate is a nucleotide, and where the
10 substrate and product are related by a cyclization or decyclization of the nucleotide.

16. The method of claim 1, where the binding partner further includes a
15 macromolecule.

17. The method of claim 1, where the binding partner further includes a
nanoparticle.

18. The method of claim 1, where the binding partner further includes a
20 quencher or an energy transfer partner.

19. The method of claim 1, the sample being supported by a sample holder, where the binding partner is linked to the sample holder.

20. The method of claim 1, where the enzyme is selected from the group consisting of kinases and phosphatases.

21. The method of claim 1, where the enzyme is selected from the group consisting of cyclases and phosphodiesterases.

22. The method of claim 1, where the substrate includes a phosphorylated polypeptide or a nonphosphorylated polypeptide.

23. The method of claim 1, where the substrate includes a cyclized nucleotide or a noncyclized nucleotide.

24. The method of claim 1, further comprising:
contacting the substrate and enzyme with a candidate compound; and
determining the ability of the candidate compound to enhance or inhibit enzyme activity by its effects on the response.

25. The method of claim 1, the specific binding being characterized by a binding coefficient, where the binding coefficient is no larger than about 10^{-8} M.

26. The method of claim 1, further comprising:

providing a sample holder having a plurality of sample sites supporting a corresponding plurality of samples; and

repeating the steps of contacting, detecting, and correlating for the plurality of

5 samples.

27. A method of detecting phosphorylation or nonphosphorylation of a polypeptide in a sample, comprising:

contacting the polypeptide with a binding partner that specifically binds to the phosphorylated polypeptide or to the nonphosphorylated polypeptide but not to both, where the binding partner includes a metal ion that is required for binding between the binding partner and the phosphorylated polypeptide or nonphosphorylated polypeptide;

detecting a response indicative of the extent of binding between the polypeptide and the binding partner without separating the bound polypeptide from the unbound polypeptide; and

correlating the response with the extent of phosphorylation or nonphosphorylation of the polypeptide, or with the activity of an enzyme that affects phosphorylation or nonphosphorylation of the polypeptide.

28. The method of claim 27, where the metal ion is a tricationic metal ion.

29. The method of claim 28, where the tricationic metal ion is selected from the group consisting of Al^{3+} , Fe^{3+} , and Ga^{3+} .

30. The method of claim 29, where the tricationic metal ion is Ga^{3+} .

31. The method of claim 28, where the binding partner further includes a dicationic metal ion.

32. The method of claim 27, where the step of detecting a response comprises:
exposing the sample to a condition capable of inducing luminescence from the sample; and

measuring a detectable luminescence response, where the detectable luminescence response is indicative of the extent of binding between the polypeptide polypeptide and the binding partner.

33. The method of claim 32, where at least one of the phosphorylated polypeptide, nonphosphorylated polypeptide, and the polypeptide is luminescent.

34. The method of claim 32, where the condition is light capable of inducing photoluminescence.

35. The method of claim 34, where the detectable luminescence response is luminescence intensity.

36. The method of claim 34, where the detectable luminescence response is luminescence polarization.

37. The method of claim 34, where the detectable luminescence response is luminescence resonance energy transfer.

38. The method of claim 32, where the condition is electrochemical energy capable of inducing electrochemiluminescence.

39. The method of claim 27, where the polypeptide includes fewer than about 50 amino acids.

40. The method of claim 27, where the binding partner further includes a macromolecule.

41. The method of claim 27, where the binding partner further includes a nanoparticle.

42. The method of claim 27, where the binding partner further includes a quencher or an energy transfer partner.

43. The method of claim 27, the sample being supported by a sample holder,
5 where the binding partner is linked to the sample holder.

44. The method of claim 27, where the binding partner binds to the phosphorylated polypeptide.

45. The method of claim 27, where the binding partner is not an polypeptide.

46. The method of claim 27, where the enzyme catalyzes addition or cleavage
of a phosphate group to or from a protein, further comprising contacting the polypeptide
with the enzyme prior to the steps of contacting, measuring, and correlating.

47. The method of claim 46, where the enzyme is a kinase.

48. The method of claim 46, where the enzyme is a phosphatase.

115
114
113
112
111
110
109
108
107
106
105
104
103
102
101
100
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

49. The method of claim 46, further comprising:
contacting the polypeptide and enzyme with a candidate compound; and
determining the ability of the candidate compound to enhance or inhibit
phosphorylation or dephosphorylation of the polypeptide by its effects on the response.

5

50. The method of claim 27, the specific binding being characterized by a
binding coefficient, where the binding coefficient is no larger than about 10^{-8} M.

51. The method of claim 27, further comprising:
providing a sample holder having a plurality of sample sites supporting a
corresponding plurality of samples; and
repeating the steps of contacting, detecting, and correlating for the plurality of
samples.

104455-0470-5594460
T92270-5594460
T15

52. A method of detecting phosphorylation or nonphosphorylation of a substrate in a sample, comprising:

contacting the substrate with a binding partner that specifically binds to the phosphorylated substrate but not to the nonphosphorylated substrate, where the binding partner is not a polypeptide;

detecting a response indicative of the extent of binding between the substrate and the binding partner without separating the bound substrate from the unbound substrate; and

correlating the response with the extent of phosphorylation or nonphosphorylation of the substrate, or with the activity of an enzyme that affects phosphorylation or nonphosphorylation of the substrate.

53. The method of claim 52, where the binding partner includes a metal ion that is required for binding between the binding partner and the substrate.

54. A method of detecting phosphorylation or nonphosphorylation of a substrate in a plurality of samples, comprising:

providing a sample holder having a plurality of discrete sample sites for supporting a corresponding plurality of samples, where each sample site includes at least one assay surface adapted to bind specifically to the phosphorylated substrate but not to the nonphosphorylated substrate;

contacting the assay surface in at least one assay site with a sample having the substrate;

detecting a response indicative of the extent of binding between the substrate and the assay surface; and

correlating the extent of binding with the extent of phosphorylation or nonphosphorylation of the substrate, or with the activity of an enzyme that affects phosphorylation or nonphosphorylation of the substrate.

55. The method of claim 54, where the sample holder is a microplate.

56. The method of claim 54, where the assay surface includes a metal ion that is required for the binding between the substrate and the assay surface.

57. The method of claim 54, where the metal ion is selected from the group consisting of Al^{3+} , Fe^{3+} , and Ga^{3+} .

58. The method of claim 54, where the step of detecting a response comprises:
exposing the sample to a condition capable of inducing luminescence from the
sample; and

measuring a detectable luminescence response, where the detectable luminescence
5 response is indicative of the extent of binding between the substrate and the binding
partner.

59. The method of claim 58, where the response is luminescence intensity.

60. The method of claim 54, where the response is absorption.

61. The method of claim 54, where the step of detecting a response is
performed without separating the bound substrate from the unbound substrate.

62. The method of claim 61, the sample holder having a bottom surface that
transmits light, where the step of detecting is performed through the bottom surface.

63. The method of claim 61, further comprising adding a blocking reagent to
reduce background prior to the step of detecting a response.

03444555-042705

64. The method of claim 54, further comprising washing the sample to remove any substrate nucleotide not bound to the assay surface prior to the step of detecting a response.

5 65. The method of claim 54, further comprising eluting the bound substrate from the assay surface prior to the step of detecting a response.

66. The method of claim 54, where the enzyme is a kinase or a phosphatase.

67. A method of detecting cyclization or noncyclization of a nucleotide in a sample, comprising:

contacting a nonradioactive nucleotide with a binding partner that specifically binds to a cyclized nucleotide or to a noncyclized nucleotide but not to both, substantially without regard to the nucleoside portion of the nucleotide;

detecting a response indicative of the extent of binding between the nucleotide and the binding partner; and

correlating the response with the extent of cyclization or noncyclization of the nucleotide, or with the activity of an enzyme that affects cyclization or noncyclization of the nucleotide.

094455-042705
10/24/05 5:54:40 PM

68. The method of claim 67, where the binding partner includes a metal ion that is required for binding between the binding partner and the cyclized nucleotide or noncyclized nucleotide.

5 69. The method of claim 68, where the tricationic metal ion is a tricationic metal ion.

70. The method of claim 69, where the tricationic metal ion is selected from the group consisting of Al^{3+} , Fe^{3+} , and Ga^{3+} .

71. The method of claim 69, where the binding partner further includes a dicationic metal ion.

72. The method of claim 67, where the step of detecting a response comprises:
exposing the sample to a condition capable of inducing luminescence from the sample; and

measuring a detectable luminescence response, where the detectable luminescence response is indicative of the extent of binding between the nucleotide and the binding partner.

73. The method of claim 72, where at least one of the cyclized nucleotide, noncyclized nucleotide, and the nucleotide is luminescent.

74. The method of claim 72, where the condition is light capable of inducing photoluminescence.

5 75. The method of claim 74, where the detectable luminescence response is luminescence intensity.

76. The method of claim 74, where the detectable luminescence response is luminescence polarization.

77. The method of claim 74, where the detectable luminescence response is luminescence resonance energy transfer.

78. The method of claim 72, where the condition is electrochemical energy capable of inducing electrochemiluminescence.

79. The method of claim 67, where the nucleotide includes an adenine or a guanine.

20 80. The method of claim 67, where the binding partner further includes a macromolecule.

81. The method of claim 67, where the binding partner further includes a nanoparticle.

82. The method of claim 67, where the binding partner further includes a
5 quencher or an energy transfer partner.

83. The method of claim 67, the sample being supported by a sample holder, where the binding partner is linked to the sample holder.

84. The method of claim 67, where the binding partner binds to the cyclized nucleotide.

85. The method of claim 67, where the binding partner is not an polypeptide.

86. The method of claim 67, where the enzyme catalyzes cyclization or decyclization of a nucleotide, further comprising contacting the nucleotide with the enzyme.

87. The method of claim 86, where the enzyme is a phosphodiesterase.

88. The method of claim 86, where the enzyme is a cyclase.

89. The method of claim 86, further comprising:
contacting the nucleotide and enzyme with a candidate compound; and
determining the ability of the candidate compound to enhance or inhibit
cyclization or decyclization of the nucleotide by its effects on the response.

5

90. The method of claim 67, where the step of detecting a response is
performed without separating the bound nucleotide from the unbound nucleotide.

91. The method of claim 67, further comprising washing the sample to remove
any nucleotide not bound to the binding partner prior to the step of measuring the
detectable luminescence response.

92. The method of claim 67, the specific binding being characterized by a
binding coefficient, where the binding coefficient is no larger than about 10^{-8} M.

93. The method of claim 67, further comprising:
providing a sample holder having a plurality of sample sites supporting a
corresponding plurality of samples; and

repeating the steps of contacting, detecting, and correlating for the plurality of
samples.

20

Pat
C1